

## Cardiovascular Effects of Combination of Perindopril, Candesartan, and Amlodipine in Hypertensive Rats

Shokei Kim, Yumei Zhan, Yasukatsu Izumi, Hiroshi Iwao

**Abstract**—The combination therapy with ACE inhibitors, angiotensin II type 1 (AT<sub>1</sub>) receptor antagonists, or calcium channel antagonists may exert more beneficial effects on cardiovascular diseases than monotherapy. Perindopril, candesartan cilexetil, or amlodipine alone or the combination of low doses of each agent was administered orally to stroke-prone spontaneously hypertensive rats (SHRSP) for 4 weeks to compare the hypotensive or cardiovascular effects. Although perindopril (2 mg/kg), candesartan cilexetil (2 mg/kg), or amlodipine (3 mg/kg) alone caused comparable hypotensive effects in SHRSP, monotherapy with perindopril or candesartan decreased left ventricular (LV) weight; mRNA levels for atrial natriuretic factor, skeletal  $\alpha$ -actin, and collagen types I and III; and aortic weight and platelet-derived growth factor- $\beta$  receptor tyrosine phosphorylation to a greater extent than monotherapy with amlodipine. Although monotherapy with a low dose (0.2 mg/kg) of perindopril or candesartan cilexetil did not significantly reduce the LV mRNA levels and aortic platelet-derived growth factor- $\beta$  receptor phosphorylation of the SHRSP, combination therapy at such a low dose normalized these parameters more potently than the use of amlodipine (3 mg/kg) alone. Although perindopril or candesartan cilexetil alone at 0.05 mg/kg did not decrease the blood pressure of the SHRSP, such a low dose of combination therapy decreased LV weight and atrial natriuretic factor mRNA levels of the SHRSP to a greater extent than amlodipine alone or amlodipine combined with perindopril or candesartan cilexetil. Our results provide evidence that suggests the combination of an ACE inhibitor and an AT<sub>1</sub> receptor antagonist may be more effective in the treatment of cardiac and vascular diseases than the combination of a calcium channel blocker with an ACE inhibitor or an AT<sub>1</sub> receptor antagonist or monotherapy with each agent. (*Hypertension*. 2000;35:769-774.)

**Key Words:** angiotensin ■ calcium ■ hypertrophy ■ platelet-derived growth factor ■ rats, stroke-prone SHR ■ antihypertensive therapy

Clinically, combination therapy with different types of antihypertensive drugs is very frequently used in hypertensive patients in an effort to achieve more beneficial effects than monotherapy and to diminish the incidence of unwanted side effects. Accumulating evidence indicates that ACE inhibitors and angiotensin (Ang) II type 1 (AT<sub>1</sub>) receptor antagonists are effective in the treatment of cardiovascular diseases or congestive heart failure.<sup>1-5</sup> Recently, in spontaneously hypertensive rats<sup>6</sup> and the (mRen-2)27 transgenic rats,<sup>7</sup> the combination of low doses of an ACE inhibitor and an AT<sub>1</sub> receptor antagonist has been shown to induce greater reductions in blood pressure and cardiac weight than monotherapy with the same or higher doses. Furthermore, the combined administration of an ACE inhibitor and an AT<sub>1</sub> receptor antagonist decreases blood pressure in normotensive volunteers<sup>8,9</sup> and improves cardiac dysfunction in patients with heart failure<sup>3,10</sup> more than either intervention alone, suggesting that combined ACE inhibitor and AT<sub>1</sub> receptor antagonist therapy may be more effective for the treatment of hypertension or heart failure than either agent alone.

In addition to blocking the renin-angiotensin system, long-acting calcium channel blockers are reported to be effective for

the treatment of cardiovascular diseases as well as hypertension.<sup>11-13</sup> Therefore, a direct comparison between calcium channel blockers and renin-angiotensin blockers, regarding the effects on cardiovascular injuries, is clinically very important. However, there is little information on the effects on cardiovascular diseases of the combined administration of a calcium channel blocker with a renin-angiotensin blocker. Furthermore, it is unclear which combination therapy is more organ protective: a calcium channel blocker combined with a renin-angiotensin blocker or an ACE inhibitor combined with an AT<sub>1</sub> receptor antagonist. In the present study, we obtained evidence that suggests the combination of an ACE inhibitor and an AT<sub>1</sub> receptor antagonist may be more effective in the treatment of cardiovascular diseases than a calcium channel blocker combined with an ACE inhibitor or an AT<sub>1</sub> receptor antagonist.

### Methods

#### Experimental Protocol

All procedures were in accordance with institutional guidelines for animal research. Stroke-prone spontaneously hypertensive rats

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From the Department of Pharmacology, Osaka City University Medical School, Abeno-ku, Osaka, Japan.

Correspondence to Shokei Kim, MD, PhD, Department of Pharmacology, Osaka City University Medical School, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan. E-mail: kims@med.osaka-cu.ac.jp

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**TABLE 1. Effects of Low Doses of Perindopril, Candesartan Cilexetil, or Amlodipine on Blood Pressure of SHRSP**

Blood Pressure, mm Hg	Vehicle	Perindopril, mg/kg		Candesartan Cilexetil, mg/kg		Amlodipine, 0.5 mg/kg
		0.05	0.2	0.05	0.2	
1 wk	224±6	218±7	204±6*	215±8	199±4*	192±5†
4 wk	221±7	216±8	196±5*	213±9	192±5*	187±7†

Blood pressure was measured at 1 and 4 weeks after the start of each drug treatment and at 4 to 5 hours after oral dosing of each drug or vehicle, because the maximal hypotensive effects of these drugs occurred at 4 to 6 hours after oral dosing. Values are mean±SEM (n=6 or 7).

\* $P<0.05$ , † $P<0.01$  vs vehicle.

(SHRSP) and control Wistar-Kyoto (WKY) rats were purchased from Japan SLC.

In preliminary experiments, low doses of perindopril (0.05 and 0.2 mg·kg<sup>-1</sup>·<sup>-1</sup>), candesartan cilexetil (0.05 and 0.2 mg·kg<sup>-1</sup>·<sup>-1</sup>), or amlodipine (0.5 mg·kg<sup>-1</sup>·<sup>-1</sup>) alone was orally administered to SHRSP once a day for 4 weeks (from 13 to 17 weeks of age). As shown in Table 1, perindopril or candesartan cilexetil alone at the dose of 0.05 mg/kg did not significantly lower the blood pressure of the SHRSP throughout the treatment. On the other hand, 0.2 mg/kg perindopril or candesartan cilexetil or 0.5 mg/kg amlodipine slightly but significantly decreased the blood pressure of the SHRSP (Table 1). Based on these preliminary data, in this work, to examine the effects of combination therapy, we used the dose of both 0.05 and 0.2 mg/kg for perindopril and candesartan cilexetil and the dose of 0.5 mg/kg for amlodipine, as described later.

In the first set of experiments, SHRSP were randomly separated into 8 groups and were orally administered (1) perindopril (2 mg/kg), (2) candesartan cilexetil (2 mg/kg), (3) amlodipine (3 mg/kg), (4) perindopril (0.2 mg/kg) and candesartan cilexetil (0.2 mg/kg), (5) perindopril (0.05 mg/kg) and candesartan cilexetil (0.05 mg/kg), (6) amlodipine (0.5 mg/kg) and perindopril (0.2 mg/kg), (7) amlodipine (0.5 mg/kg) and candesartan cilexetil (0.2 mg/kg), or (8) vehicle (0.5% carboxymethylcellulose solution). All drugs were administered to SHRSP via gastric gavage once a day for 4 weeks (from 13 to 17 weeks of age). The systolic blood pressure of the conscious rats was measured with the tail-cuff method at 4 to 5 hours after oral dosing, when these drugs exhibited the maximal hypotensive effects. After the treatment, the rats were decapitated, and the heart and the thoracic aorta were immediately excised. The left ventricle was separated from the atria and the right ventricle, the thoracic aorta was carefully dissected from adherent fat and connective tissues, and they were weighed, immediately frozen in liquid nitrogen, and stored at -80°C until use.

In the second set of experiments, to investigate the possible contribution of bradykinin to the effects of perindopril, SHRSP were separated into 3 groups and administered (1) vehicle, (2) perindopril (2 mg·kg<sup>-1</sup>·<sup>-1</sup>), or (3) perindopril (2 mg·kg<sup>-1</sup>·<sup>-1</sup>) combined with the bradykinin B<sub>2</sub> receptor antagonist Hoe140 at a dose of 300 µg·kg<sup>-1</sup>·<sup>-1</sup>. Hoe140 was subcutaneously infused to rats via an osmotic minipump (Alza Corp); such a dose of Hoe140 has been generally used to block ACE inhibitor-induced bradykinin action in vivo<sup>14</sup> and is shown to completely block the vasodepressor effect of exogenous bradykinin in rats.<sup>15</sup>

#### RNA Preparation and Northern Blot Analysis

All procedures were performed as described in detail in our previous reports.<sup>16,17</sup> In brief, total RNA was isolated from the individual left ventricle according to the acid guanidinium thiocyanate/phenol/chloroform method, and 20 µg of total RNA samples was subjected to 1% agarose gel electrophoresis and transferred to a nylon membrane. Hybridization was performed with <sup>32</sup>P-dCTP-labeled cDNA probe for atrial natriuretic factor (ANF), collagen type I, collagen type III, or GAPDH or <sup>32</sup>P-ATP-labeled oligonucleotide probe complementary to skeletal  $\alpha$ -actin cDNA.<sup>16</sup> The densities of an individual mRNA band were measured with a bioimaging analyzer (BAS-2000; Fuji Photo Film Co).

#### Immunoprecipitation and Western Blot Analysis

The method of immunoprecipitation and Western blot analysis has been described in detail in our previous reports.<sup>18,19</sup> In brief, aortic tissues were homogenized in lysis buffer (20 mmol/L HEPES, pH 7.2, 25 mmol/L NaCl, 2 mmol/L EGTA, 50 mmol/L NaF, 1 mmol/L Na<sub>2</sub>VO<sub>4</sub>, 25 mmol/L  $\beta$ -glycerophosphate, 0.2 mmol/L dithiothreitol, 1 mmol/L PMSF, 60 µg/mL aprotinin, 2 µg/mL leupeptin, and 0.1% Triton X-100), sonicated, and centrifuged to obtain the supernatant. Aortic protein extracts (250 µg) were preabsorbed with protein A-Sepharose or protein G-Sepharose and were incubated with rabbit polyclonal anti-platelet-derived growth factor (PDGF)- $\beta$  receptor antibody (Santa Cruz Biotechnology, Inc), rabbit polyclonal anti-PDGF- $\alpha$  receptor antibody (Santa Cruz Biotechnology, Inc), or sheep polyclonal anti-epidermal growth factor (EGF) receptor antibody (GIBCO BRL). The immunocomplexes were precipitated with protein A-Sepharose for anti-PDGF- $\alpha$  or - $\beta$  receptor antibody or with protein G-Sepharose for anti-EGF receptor antibody. The immunoprecipitates were boiled in Laemmli's sample buffer and centrifuged, and the resulting supernatants were electrophoresed onto 8% SDS-polyacrylamide gel and transferred to Hybond-PVDF membranes (Amersham Life Sciences). The membranes were immunoblotted with mouse monoclonal anti-phosphotyrosine antibody (Upstate Biotechnology). Immunocomplexes were visualized by using the enhanced chemiluminescence (ECL) method (Amersham). The densities were measured with the public domain National Institutes of Health IMAGE program. After the previous antibody was stripped off, the membranes were again immunoblotted with the anti-PDGF- $\beta$  receptor antibody, anti-PDGF- $\alpha$  receptor antibody, or anti-EGF receptor antibody as described.

#### Statistics

Results were expressed as mean±SEM. Statistical significance was determined with 1-way ANOVA followed by Duncan's multiple range test. Differences were considered statistically significant at a value of  $P<0.05$ .

#### Results

##### Effects of Combination Therapy on Blood Pressure of SHRSP

The hypotensive effects in SHRSP of low doses of each drug, used for combination therapy, are shown in Table 1. As shown in Table 2, the administration of the combination of perindopril and candesartan cilexetil (0.2 mg/kg each) produced hypotensive effect comparable to that of perindopril, candesartan cilexetil, and amlodipine alone (2, 2, and 3 mg/kg, respectively). Although 0.05 mg/kg perindopril or candesartan cilexetil alone did not lower the blood pressure of the SHRSP (Table 1), their combination significantly lowered the blood pressure. On the other hand, the hypotensive effects of the combination of 0.5 mg/kg amlodipine and 0.2 mg/kg perindopril or candesartan cilexetil were significantly lesser

TABLE 2. Blood Pressure, Body Weight, Left Ventricular Weight, and Aortic Weight in Each Group of SHRSP

Parameter	Vehicle	Therapy, mg · kg <sup>-1</sup> · d <sup>-1</sup>			Combination Therapy, mg · kg <sup>-1</sup> · d <sup>-1</sup>			
		Pe (2)	Ca (2)	Am (3)	Pe (0.2) + Ca (0.2)	Pe (0.05) + Ca (0.05)	Am (0.5) + Pe (0.2)	Am (0.5) + Ca (0.2)
BP, mm Hg at 1 wk	221±5	147±9*	153±4*	145±4*	160±3*	189±3*†	181±4*†	180±5*†
BP, mm Hg at 4 wk	217±6	157±4*	155±4*	151±3*	148±4*	176±3*†	176±4*†	172±2*†
BW, g	295±5	295±5	306±5	292±3	301±5	297±3	299±5	290±5
LV/BW, mg/g BW	2.99±0.04	2.46±0.04*†	2.38±0.04*†	2.76±0.04*	2.40±0.02*†	2.59±0.03*†	2.77±0.04*‡	2.70±0.02*‡
Ao/BW, mg/100 g BW	19.3±0.6	15.6±0.5*†	16.5±0.4*†	18.7±0.5	16.3±0.5*†	18.5±0.7	19.0±0.7	18.6±0.7

Pe indicates perindopril; Ca, candesartan cilexetil; Am, amlodipine; BP, blood pressure; BW, body weight; LV/BW, left ventricular weight corrected for body weight; and Ao/BW, aortic weight corrected for body weight. Values are mean±SEM (n=7 or 8).

\**P*<0.05 vs vehicle.

†*P*<0.05 vs Am (3).

‡*P*<0.05 vs Pe (0.05)+Ca (0.05).

than those of the combination of perindopril and candesartan cilexetil (0.2 mg/kg each) (*P*<0.01) and were comparable to those of the combination at a dose of 0.05 mg/kg each. Thus, the combination of an ACE inhibitor and an AT<sub>1</sub> receptor antagonist more potently decreased the blood pressure of the SHRSP than did the combination of a calcium channel blocker with an ACE inhibitor or an AT<sub>1</sub> receptor antagonist.

#### Effects of Combination Therapy on Left Ventricular Weight and Gene Expression of SHRSP

As shown in Table 2, 2 mg/kg perindopril or candesartan cilexetil alone, and their combination (0.2 mg/kg each), decreased the left ventricular weight of the SHRSP to a greater extent than 3 mg/kg amlodipine alone (*P*<0.01). Notably, even the combination of 0.05 mg/kg perindopril and candesartan cilexetil, despite the less hypotensive effects, reduced left ventricular weight more potently than amlodipine alone (*P*<0.01). Furthermore, the combination of 0.5 mg/kg amlodipine with 0.2 mg/kg perindopril or candesartan

cilexetil, which produced a less hypotensive effect than the use of 3 mg/kg amlodipine alone, reduced left ventricular weight as much as the use of amlodipine alone.

We examined the effects of combination therapy on cardiac hypertrophy and remodeling-associated genes, including fetal phenotype of genes (ANF and skeletal  $\alpha$ -actin) and collagen types I and III. These gene expressions are significantly enhanced in SHRSP compared with control normotensive WKY rats, as we previously demonstrated.<sup>20</sup> In our preliminary experiments, we examined the effects of monotherapy of 0.2 mg/kg perindopril, 0.2 mg/kg candesartan cilexetil, or 0.5 mg/kg amlodipine on the cardiac mRNAs of the SHRSP and found that such low doses of each drug failed to reduce the mRNA levels (data not shown). As shown in Figure 1, high doses of monotherapy drugs or low doses of combination therapy drugs that we examined all significantly decreased left ventricular mRNA levels for ANF, skeletal  $\alpha$ -actin, collagen type I, and collagen type III. Interestingly, perindopril or candesartan cilexetil (2 mg/kg) or their combination (0.2 mg/kg each) decreased left ventricular ANF,

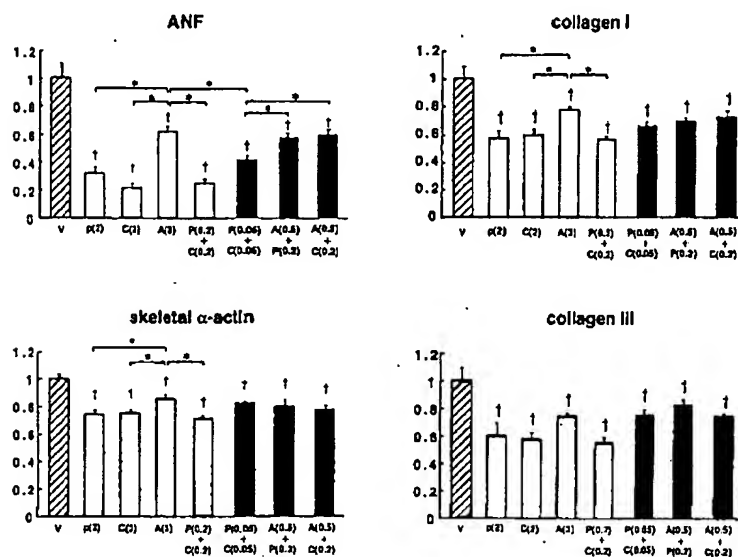


Figure 1. Left ventricular mRNA levels for ANF, skeletal  $\alpha$ -actin, collagen type I, and collagen type III in each group of SHRSP. V indicates vehicle; P(2), 2 mg/kg perindopril; C(2), 2 mg/kg candesartan cilexetil; A(3), 3 mg/kg amlodipine; P(0.2)+C(0.2), 0.2 mg/kg perindopril and 0.2 mg/kg candesartan cilexetil; P(0.05)+C(0.05), 0.05 mg/kg perindopril and 0.05 mg/kg candesartan cilexetil; A(0.5)+P(0.2), 0.5 mg/kg amlodipine and 0.2 mg/kg perindopril; A(0.5)+C(0.2), 0.5 mg/kg amlodipine and 0.2 mg/kg candesartan cilexetil. Four groups of SHRSP (open column) had similar blood pressures, as shown in Table 2. Three groups of SHRSP (filled column) had similar blood pressures, as shown in Table 2. Values are mean±SEM (n=7 or 8). The mean value of V (vehicle-treated group) is represented as 1. †*P*<0.05 vs V (vehicle). \**P*<0.05.

TABLE 3. Tyrosine Phosphorylation of Aortic PDGF and EGF Receptors in 17-Week-Old SHRSP and WKY Rats

Group	PDGF- $\beta$ Receptor		PDGF- $\alpha$ Receptor		EGF Receptor	
	Phospho Tyr	Protein Level	Phospho Tyr	Protein Level	Phospho Tyr	Protein Level
SHRSP	1.85 $\pm$ 0.11*	1.16 $\pm$ 0.07	1.25 $\pm$ 0.12	1.15 $\pm$ 0.09	0.98 $\pm$ 0.09	0.89 $\pm$ 0.05
WKY Rats	1 $\pm$ 0.04	1 $\pm$ 0.05	1 $\pm$ 0.07	1 $\pm$ 0.08	1 $\pm$ 0.08	1 $\pm$ 0.06

Phospho Tyr indicates tyrosine phosphorylation of each receptor in aorta, estimated with immunoprecipitation followed by Western blot analysis as described in the text. Values are mean $\pm$ SEM (n=6 or 7). The mean value of WKY rats is represented as 1.

\* $P<0.01$  vs WKY rats.

skeletal  $\alpha$ -actin, and collagen type I mRNA levels to a greater extent than did 3 mg/kg amlodipine alone. Furthermore, although the combination of 0.05 mg/kg perindopril and candesartan cilexetil had a less hypotensive effect than 3 mg/kg amlodipine alone and had similar hypotensive effects as 0.5 mg/kg amlodipine combined with perindopril or candesartan cilexetil, this combination decreased left ventricular ANF mRNA levels to a greater extent than amlodipine alone or the combination with perindopril or candesartan cilexetil.

#### Aortic PDGF and EGF Receptors in SHRSP

As shown in Table 3, aortic PDGF- $\beta$  receptor tyrosine phosphorylation in the SHRSP was significantly increased by 1.85-fold ( $P<0.01$ ) compared with the control WKY rats, whereas there was no significant difference in aortic PDGF- $\beta$  receptor protein levels between the SHRSP and WKY rats, indicating that aortic PDGF- $\beta$  receptor activation is enhanced in SHRSP compared with WKY rats. On the other hand, there was no statistically significant difference between the SHRSP and WKY rats with respect to aortic PDGF- $\alpha$  receptor tyrosine phosphorylation or protein levels or EGF receptor tyrosine phosphorylation or protein levels (Table 3). Perindopril or candesartan cilexetil alone (2 mg/kg), and their combination (each 0.2 mg/kg), significantly decreased not only aortic weight (Table 2) but also aortic PDGF- $\beta$  receptor tyrosine phosphorylation of the SHRSP to a comparable degree, whereas other drug treatments did not significantly affect their parameters (Figure 2). On the other hand, aortic PDGF- $\beta$  receptor protein levels were not significantly affected by any drug treatments (Figure 2).

#### Effect of Hoe140 on Perindopril-Treated SHRSP

Hoe140, a bradykinin B<sub>2</sub> receptor antagonist, had no effect on the mentioned effects of perindopril on blood pressure; left ventricular weight; aortic weight; left ventricular mRNAs for ANF, skeletal  $\alpha$ -actin, collagen type I, and collagen type III; and aortic PDGF- $\beta$  receptor tyrosine phosphorylation in SHRSP (data not shown).

#### Discussion

Combination therapy is very frequently used in hypertensive patients. Furthermore, recent experimental or clinical evidence suggests that combination therapy of an ACE inhibitor and an AT<sub>1</sub> receptor antagonist is useful for the treatment of hypertension and cardiac hypertrophy or to improve cardiac function.<sup>6,8-10,21,22</sup> However, the effects of the combination of calcium channel blockers with an ACE inhibitor or an AT<sub>1</sub>

receptor antagonist are poorly understood. Furthermore, it is unclear which combination therapy is more effective: the combination of an ACE inhibitor with an AT<sub>1</sub> receptor antagonist or the combination of a calcium channel blocker with a renin-angiotensin blocker. Therefore, in the present work, we compared the effects of combination therapy on cardiac hypertrophy and gene expression and aortic growth factor receptor activation.

Our present observations provide evidence that the combination of an ACE inhibitor with an AT<sub>1</sub> receptor antagonist produced hypotensive effects that were greater than those provided by the combination of a calcium channel blocker with an ACE inhibitor or an AT<sub>1</sub> receptor antagonist, which supports previous experimental and clinical data<sup>6-9</sup> indicating that the combination of an ACE inhibitor and an AT<sub>1</sub> receptor antagonist may be useful for the treatment of hypertension. Cardiac hypertrophy is characterized not only by the increase in myocyte size but also by gene reprogramming such as the upregulation of fetal genes and extracellular matrix genes.<sup>23,24</sup> Thus, it is essential for the estimation of cardiac hypertrophy to examine the effects on cardiac gene expressions. However, the effects of combination therapy on cardiac gene expression have not been examined in previous reports.<sup>6,7,22</sup> Therefore, here, we investigated cardiac gene expressions and found that the combination of low doses of an ACE inhibitor and an AT<sub>1</sub> receptor antagonist (0.2 mg/kg each) decreased cardiac mRNA levels for ANF, collagen type I, and skeletal  $\alpha$ -actin more potently than amlodipine alone. Furthermore, the combination of 0.05 mg/kg perindopril and candesartan cilexetil, whose hypotensive effects were similar to those of low doses of amlodipine combined with perindopril or candesartan cilexetil and were lesser than those of high doses (3 mg/kg) of amlodipine alone (Table 2), decreased left ventricular weight and ANF mRNA levels more potently than combination therapy or monotherapy with amlodipine. Thus, the combination of an ACE inhibitor and an AT<sub>1</sub> receptor antagonist may be more effective in not only regression of cardiac hypertrophy but also normalization of cardiac gene reprogramming than monotherapy or combination therapy with a calcium channel blocker.

In addition to the heart, vascular tissues are another important target for antihypertensive drugs. Previous work on the combination of an ACE inhibitor and an AT<sub>1</sub> receptor antagonist have not examined the effect on vascular injury.<sup>6,7,22</sup> Recent in vitro reports on cultured vascular smooth muscle cells indicate that PDGF and EGF receptors play a major role in vascular smooth muscle cell growth<sup>25</sup> and that the growth effect of Ang II is due to transactivation of EGF

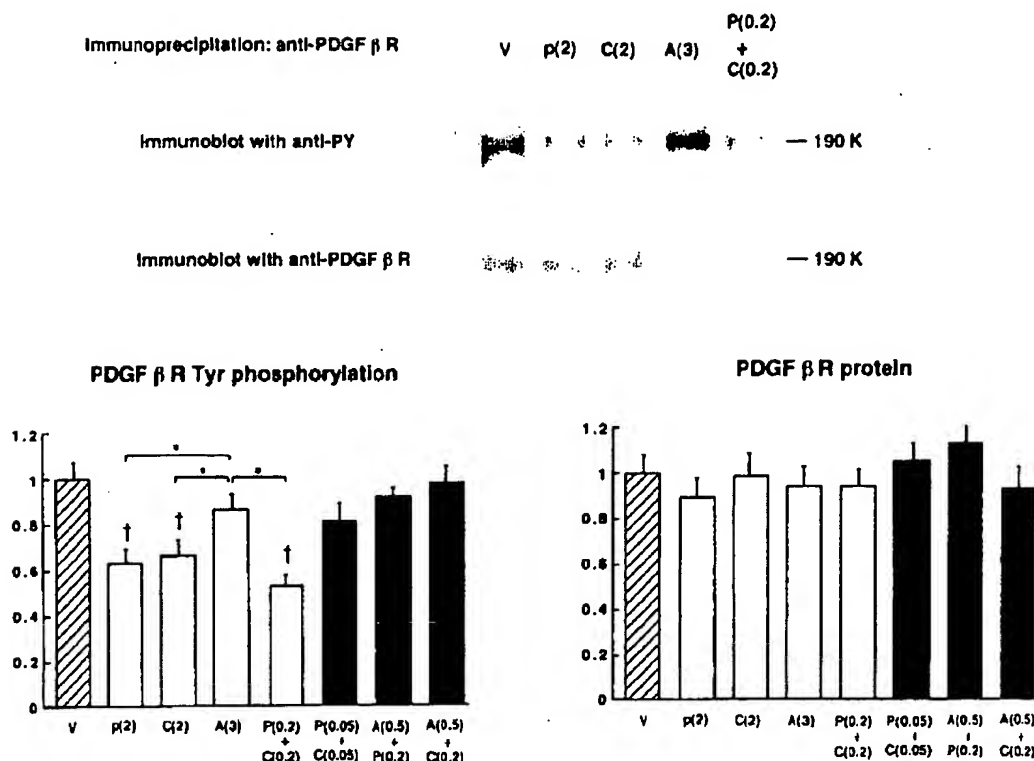


Figure 2. Aortic PDGF- $\beta$  receptor (PDGF  $\beta$  R) tyrosine phosphorylation and protein levels in each group of SHRSP. Aortic proteins were immunoprecipitated with anti-PDGF- $\beta$  receptor antibody (anti-PDGF  $\beta$  R), followed by immunoblotting with anti-phosphotyrosine antibody (anti-PY) or anti-PDGF- $\beta$  receptor antibody (anti-PDGF  $\beta$  R). Left, PDGF- $\beta$  receptor tyrosine (R Tyr) phosphorylation. Right, PDGF- $\beta$  receptor protein levels. Four groups of SHRSP (open column) had similar blood pressures, as shown in Table 2. Three groups of SHRSP (filled column) had similar blood pressures, as shown in Table 2. Abbreviations are as in the legend to Figure 1. Values are mean  $\pm$  SEM ( $n=7$  or 8). The mean value of V (vehicle-treated group) is represented as 1.  $\dagger P<0.05$  vs V (vehicle).  $*P<0.05$ .

receptor<sup>26</sup> or PDGF receptor<sup>27</sup> via AT<sub>1</sub> receptor. However, it is still unclear whether the activation of EGF or PDGF receptors by Ang II can occur in an in vivo situation. In the present work, we obtained the first evidence that vascular PDGF- $\beta$  receptor tyrosine phosphorylation, but not PDGF- $\alpha$  receptor or EGF receptor tyrosine phosphorylation, was enhanced in hypertensive rats and that this enhanced PDGF- $\beta$  receptor activation was at least in part mediated by AT<sub>1</sub> receptor, as shown by the significant inhibitory effects of perindopril and candesartan cilexetil but not amlodipine. Interestingly, although 0.2 mg/kg perindopril or candesartan cilexetil alone failed to suppress aortic PDGF- $\beta$  receptor tyrosine phosphorylation, their combination significantly decreased aortic PDGF- $\beta$  receptor tyrosine phosphorylation as much as 2 mg/kg of each agent alone. Taken together with the fact that the PDGF- $\beta$  receptor plays a central role in vascular smooth muscle cell proliferation and migration in vivo,<sup>28-30</sup> these findings support the notion that combination therapy with an ACE inhibitor and an AT<sub>1</sub> receptor antagonist may be useful for the treatment of vascular remodeling.

In the present work, either bradykinin accumulation<sup>14</sup> or AT<sub>2</sub> receptor<sup>31</sup> might be responsible for the beneficial effects of the combination of low doses of perindopril and candesartan cilexetil. Our results show that treatment with Hoe140 did

not affect the cardiac and aortic effects of perindopril. This observation suggests that there was no contribution of bradykinin B<sub>2</sub> receptor to the actions of perindopril under our experimental conditions, although the possible importance of bradykinin cannot be completely ruled out.<sup>32,33</sup> Furthermore, differing from an AT<sub>1</sub> receptor antagonist alone, the combination with an ACE inhibitor is shown to suppress plasma Ang II elevation induced by AT<sub>1</sub> receptor antagonist,<sup>6</sup> indicating that AT<sub>2</sub> receptor activation resulting from AT<sub>1</sub> receptor antagonist treatment is inhibited by the combination with an ACE inhibitor. Thus, the mechanism of greater effectiveness with the combination of an ACE inhibitor and an AT<sub>1</sub> receptor antagonist that was observed in the present work seems to be due to a more potent inhibition of Ang II-mediated AT<sub>1</sub> receptor activation itself rather than to bradykinin accumulation or AT<sub>2</sub> receptor activation. Thus, in vivo studies with an ACE inhibitor alone or an AT<sub>1</sub> receptor antagonist alone may underestimate the role of the renin-angiotensin system in hypertension and in cardiovascular hypertrophy and remodeling.

In conclusion, our present observations show that the combination of low doses of an ACE inhibitor and an AT<sub>1</sub> receptor antagonist suppresses cardiac hypertrophy-related gene reprogramming and aortic PDGF- $\beta$  receptor activation in SHRSP.

Our findings further extend the notion that the combination of an ACE inhibitor and an AT<sub>1</sub> receptor antagonist may be more effective in the treatment of hypertension and cardiovascular diseases than monotherapy.<sup>6-9,21,22</sup> Furthermore, we propose that the combination of an ACE inhibitor with an AT<sub>1</sub> receptor antagonist may be more effective in the treatment of cardiovascular hypertrophy and remodeling than the combination with a calcium channel blocker.

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